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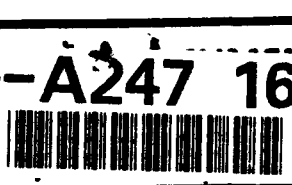

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6. AUTHOR(S)
Dr Philip R. Vulliet

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Department of Veterinary Pharmacology
and Toxicology
School of Veterinary Medicine
University of California
Davis, CA 95616

8. PERFORMING ORGANIZATION REPORT NUMBER
AFOSR-TR- 92 0085

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Dr Genevieve M. Haddad
AFOSR/NL
Building 410
Bolling AFB DC 20332-6448

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13. ABSTRACT (Maximum 200 words)

This project investigated the cellular, molecular and biochemical mechanisms that control the response of the rat adrenal gland to physiological stimulation. We found that the responsivity of this tissue will change following specific treatments including chronic treatment of rats with insulin and acute depolarization of the cells with KCl. The alternation in adrenal reactivity appears to be directly correlated with the cellular concentrations of catecholamine neurotransmitters. This past year has been spent investigating the biochemical mechanisms that may be responsible for this change in bioreactivity. Since the most important biochemical mechanism contr lling cellular function is protein phosphorylation, we focussed on this method of control. We have identified a novel protein kinase activity that phosphorylates both tyrosine hydroxylase and synapsin at a unique site. Most recent research has involved the phosphorylation of other structural proteins by this proline-directed protein kinase. In addition, we have examined the role protein kinase C in regulating the response of PC12 cells to a variety of growth factors in culture.

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FINAL TECHNICAL REPORT

BIOCHEMICAL MECHANISMS CONTROLLING BIOREACTIVITY OF ADRENAL CHROMAFFIN CELLS

8/1/88 to 12/31/90
USAFOSR Project #88-NL-006

1. SUMMARY:

This project investigated the cellular, molecular and biochemical mechanisms that control the response of the rat adrenal gland to physiological stimulation. We found that the responsivity of this tissue will change following specific treatments including chronic treatment of rats with insulin and acute depolarization of the cells with KCl. The alteration in adrenal reactivity appears to be directly correlated with the cellular concentrations of catecholamine neurotransmitters. Previous research has demonstrated that cells that demonstrate altered reactivity have characteristic morphological changes that return to normal in parallel with the glands return to normoreactivity.

This past year has been spent investigating the biochemical mechanisms that may be responsible for this change in bioreactivity. Since the most important biochemical mechanism controlling cellular function is protein phosphorylation, we focussed on this method of control. We have identified a novel protein kinase activity that phosphorylates both tyrosine hydroxylase and synapsin at a unique site. Most recent research has involved the phosphorylation of other structural proteins by this proline-directed protein kinase. In addition, we have examined the role protein kinase C in regulating the response of PC12 cells to a variety of growth factors in culture.

2. RESEARCH OBJECTIVES:

1. Optimize conditions to provide maximal alterations in the bioreactivity of the adrenal medulla.

2. Evaluate disassociated chromaffin cells isolated from animals exhibiting altered reactivity.

3. Compare localization (compartmentalization) and activity of protein kinases and their substrates in control tissues and adrenal glands demonstrating altered reactivity or from disassociated cells derived from these tissues.

4. Quantitate the biosynthesis and number of chromaffin granules, catecholamine content and evaluate the morphological characteristics of adrenal chromaffin vesicles exposed to treatments that alter the bioreactivity.

5. Purify and evaluate chromaffin vesicles from the adrenal medullary cells with respect to catecholamine content, protein content, protein kinase substrate content

and ability to sequester calcium.

6. Quantitate the degree of phosphorylation and specific sites of phosphorylation of functionally significant proteins such as tyrosine hydroxylase, protein III, Protein F1 and vesicle associated phosphoproteins from control, hypo- and hyper-reactive adrenal glands.

3. STATUS OF RESEARCH:

The research efforts described in this report covers the period from 8/1/88 to 12/31/90 and has focussed on investigating the biochemical mechanisms that control the response of the cell. During this period, we have: a) investigated the role of protein kinase C in regulating the response of PC12 cells to growth factors, b) identified a novel protein kinase activity found in PC12 cells, c) established that this kinase was activated by treatment with NGF, d) examined other proteins and peptides as substrates of this novel protein kinase activity, e) identified the specific sequence recognition requirements for this kinase, f) developed techniques for transfection of tissue culture cells. Each of these techniques will be discussed individually below.

A. Identification of the role of protein kinase C in mediating the effects of growth factors.

We recently reported that the neurite inducing action of NGF on PC12 cells was decreased when sphingosine, a specific inhibitor of protein kinase C was given at the same time. This inhibitory effect could be reversed by adding TPA, a competitive agonist for the same site on PK C. further studies have been performed to elucidate the site at which PK C is acting.

B. Identification of a novel protein kinase activity.

Previous work had identified and sequenced a novel site of phosphorylation on tyrosine hydroxylase, the rate limiting enzyme in the biosynthesis of the catecholamine neurotransmitters. This site did not have any of the sequences associated with the classical protein kinases. Using this sequence, synthetic peptide that was identical with the first 15 amino acids of tyrosine hydroxylase was produced. It was established that this peptide would serve as a substrate for this novel kinase, allowing the development of an assay for this kinase activity.

C. Partial purification of the novel protein kinase.

Using this assay for activity the elution of this kinase activity was examined from several chromatographic media, including: DEAE cellulose, carboxymethylcellulose, heparin sepharose, cellulose phosphate, Mono Q, and a variety of gel permeation media. It was found that this kinase activity eluted as a protein with molecular mass of 60 kDa. Techniques for the obtaining this kinase activity with an approximate 5% purity have been obtained so far.

D. Examination of other proteins and peptides as substrates of this novel protein kinase activity.

Once this novel kinase activity had been isolated from other known proteins kinases, other proteins were investigated as possible substrates for this kinase. Histone H1, glycogen synthase, synapsin, tyrosine hydroxylase, and the regulatory subunit of cyclic AMP dependent protein kinase have all been identified as in vitro substrates.

E. Identified the specific sequence recognition requirements for this kinase.

Since the site phosphorylated on tyrosine hydroxylase did not have any basic amino acid residues adjacent to it (a requirement for all of the other known classical protein kinases), the sequence specificity of this kinase was investigated using a series of selected synthetic peptides. With these peptides, the activity of this kinase was found to be dependent upon the presence of a proline residue on the carboxyl terminal side of the phosphorylated serine/threonine residue. Therefore, we elected to name this protein kinase "proline -directed protein kinase" to identify it by it's substrate recognition sequence selectivity.

F. Established that this kinase was activated by treatment with NGF.

Using the assay developed for the novel protein kinase, PC12 were treated with NGF for varying periods of time. The novel kinase activity was found to be increased at 30 sec following addition of NGF. The activity of this kinase was maximal at 1 to 2 min following NGF treatment.

G. Developed techniques for transfection of tissue culture cells.

Using a DNA construct coding for TH and its promotor region and the genticin selection region, NIH 3T3 cells have been transfected using the calcium precipitation technique. The cells are currently being screened for stable transformants and to evaluate the effects of increased levels of TH upon cellular function.

4. WRITTEN PUBLICATIONS:

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5. PROFESSIONAL PERSONNEL:

Philip R. Vulliet, Ph.D., DVM - 50% time

Ruedi Braun, Ph.D., - 100% time

Denise Carbonaro, M.S. - 50% time

Kris Kulp, B.S. - 50% time

Fred Hall, Ph.D., - 100% time

Jay Mazer, M.S. - 100% time

Jeff Mitchell, M.S. - 50% time

Janice Streisand, B.S. - 50% time

Mitch Halloran, B.S. - 100

6. PROFESSIONAL INTERACTIONS:

P.R. Vulliet, J.P. Mitchell, R. Braun and F.L. Hall. Identification of a Novel Proline-directed Protein Kinase that Phosphorylates Tyrosine Hydroxylase. Presented at the Adrenal Chromaffin Cell as a Neuronal Model: From Basic to Clinical Aspects. Jerusalem, Israel, June 2, 1989

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Breckenridge, Colorado, January, 1989

R.K. Braun, D.K. Getman, F.L. Hall. Tissue Distribution of a Novel Growth Factor-Sensitive Protein Kinase. Presented at the annual meeting of the Western Pharmacology Society, Breckenridge, Colorado, January, 1989

P.R. Vulliet, F.L. Hall, J.P. Mitchell and D. G. Hardie. Phosphorylation of the N-terminal Region of Tyrosine Hydroxylase. Presented at the annual meeting of the Society for Neuroscience, Toronto, November, 1988